**LEARNING OBJECTIVES:**

Introduction to Cell Biology:

1. Describe the basic architecture and specialized functions of eukaryotic cells

Plasma membrane separates cell from outside environment

Has 2 main components, nucleus and cytosol

Nucleus contains the DNA

Cytosol contains the organelles

Has a cytoskeleton for structure and support

Typical human cell is ~10µm in diameter

1. Describe and compare basic techniques used to study cells and subcellular organells

Light microscopy:

1. resolving power ~0.2 µm
2. Can observe live cells

Electron microscopy:

1. Shorter wavelength radiation used than light microscope
2. Requires cells to be fixed
3. Can resolve objects to ~1 nm

Centrifugation:

1. Separates cellular components based on mass
2. Usually destroys the cells

Cell Culture:

1. Can study the interactions of living cells as they grow and differentiate
2. Useful for determining function of genes and proteins as they can be knocked out or over expressed in the culture and the effects observed
3. Describe the structure and function of intracellular organelles

Smooth Endoplasmic Reticulum (Smooth ER)

1. No ribosomes so appearance under a microscope is of a smooth membrane hence the name
2. Synthesizes steroids, cholesterol and triglycerides (Lipid Metabolism)
3. Detoxifies drugs using Cytochrome p450 enzymes

Rough Endoplasmic Reticulum (Rough ER)

Many attached ribosomes giving the appearance of a beaded membrane

Protein synthesis and carbohydrate addition occur here

Continuous with nuclear envelope

Prevalent in cells with a high secretory load as the rough ER functions in secretory vesicles formation

Golgi Apparatus (Golgi)

Site of post-translational protein modifications (Rough ER synthesizes, golgi modifies)

Actually forms the final secretory vesicle through budding off the golgi membrane

Comprises a convex side (cis, closest to rough ER, entry phase) of vesicles, flattened slightly curved sacs (medial) and a concave (trans, exit phase) of vesicles recently formed

Mitochondria

Major site of ATP synthesis

Outer membrane is smooth, inner membrane has multiple cristae (folds)

Inter-membrane space (between outer and inner membranes)

Matrix (the lumen of the inner membrane, does not contact the outer membrane)

Averages about ~0.5-1µm in diameter but can be larger

Peroxisome

Membrane bound

Consumes oxygen and produces hydrogen peroxide a toxic compound

Catalse decomposes the peroxide into water and oxygen

Active in the catabolism of long chain fatty acids

~0.2-1µ in diameter

Lysosome

Membrane bound

Packed with degradative enzymes

Actively transports H+ into lumen reducing its pH to ~5

Cellular garbage disposal

Cytoplasmic Inclusions

Little or no metabolic activity

Not membrane bound

Ex. Glycogen and lipid droplets

1. Explain the material transport and the process of phagocytosis and pinocytosis

Nuclear Pores (Gated Transport)

1. Allows passage of certain materials between cytoplasm and nucleus
2. Proteins destined for nucleus are encoded with specific recognition sequences
3. Selective gates but allow diffusion of smaller molecules

Trans-membrane Transport

1. Transport molecules from cytoplasm into ER lumen, mitochondria or peroxisomes
2. Protein “translocaters” directly perform this function

Vesicular Transport

1. Transfer proteins from ER to golgi
2. Membrane bound irregularly shaped or spherical balls
3. Formed from donor compartment (ER) and fuse with recipient compartment (golgi)

Endocytosis

1. The process by which cells ingest substances from the outside
2. If a large vesicle is formed this is phagocytosis
3. If a small vesicle is formed this is pinocytosis

\*\*Autophagy- a special type of phagocytosis where a cell digests its own non-functional or damaged organelles/other structures

1. All material that a cell endocytosed eventually fuses with a lysosome for degradation
2. Describe similarities and differences between apoptosis and necrosis

|  |  |  |
| --- | --- | --- |
| Type of Cell Death | Similarities | Differences |
| Necrosis | Cell death | Uncontrolled  Disorganized  Cellular contents released |
| Apoptosis | Programmed death  Characteristic changes in cell  Blebbing  Cell shrinkage  Chromatin condenses irreversibly (pyknosis)  Apoptotic body formation  No inflammation  Signals sent to phagocytes to consume |

Introduction to Proteins Part 1:

1. Describe the most common types of chemical bonds in biochemical systems
2. Covalent
3. Hydrogen
4. Ionic

\*\* Van Der Waals Interactions (not really bonds per se)

1. Explain how water can affect chemical bonds
2. Non-covalent interactions/bonds are susceptible to the aqueous environment (water)
3. Water forms solvent shells (water molecules surround charged molecules or polar atoms because water itself is polar and wants to interact)
4. Water has a rapidly fluctuating hydrogen bond network which allows other polar molecules to interact with itself
5. Water also excludes non-polar molecules causing them to form aggregates (lipid membranes) this occurs because it is energetically favorable
6. Define strong and weak acids and bases
7. Strong acids/bases completely ionize in water solutions to form H+/OH- respectively and a very weak base/acid like Cl- or Na+
8. Weak acids/bases only partially ionize in water so they have an equilibrium formed with their conjugate base/acid
9. Weak acids produce strong conjugate bases and vise-versa
10. Define pH, equilibrium constants and pKa
11. pH-- the logarithmic measure of the concentration of H+ ions in solution

\*\* Defined as the –log[H+]

1. Equilibrium constant--the ratio of products to reactants when there is no net change in either
2. pKa-- the pH at which the conjugate acid concentration equals that of the conjugate base

\*\*this is where buffering is most effective

1. Explain the Henderson-Hasselbalch equation and the relationship between pH and the ratio of acid to base
2. This equation states that the pH of a buffer can be determined only by knowing the ratio of its acid and conjugate base
3. This equation shows that pH will equal pka when conjugate acid = conjugate base
4. Describe the properties of a good biological buffer
5. Resists changes in pH
6. pKa ~7 (human blood optimal pH ~7.4)
7. Define a Zwitterion and what occurs to it at different pHs
8. An molecule that exists as a uncharged ion (amino acids are protonated + on the N terminus and deprotonated – on the C terminus)
9. At low pH (high H+) both groups become protonated
10. At high pH (low H+) both groups become deprotonated
11. Write the general structure for amino acids and indicate the alpha carbon

H

|

+H3N---C---COO- \*\* alpha carbon

|

R

1. Distinguish between L and D configurations of alpha amino acids
2. Picture tetrahedral arrangement, place hydrogen in back, other 3 groups now face the plane of this page, if rotation is clockwise it is R (right, normal for clocks) but UNNATURAL in an amino acid!!
3. If rotation is counterclockwise it is L (PROPER, for amino acids)

\*\* All amino acids are L

1. Divide the amino acids into groups based on their side chain characteristics as well as the 1 and 3 letter abbreviations for each

Don’t be that lazy….just look these up ☺

Introduction to Proteins Part 2

1. Describe the mechanism of chemical bonding between two amino acids (peptide bond)
2. Alpha carbon gets bonded to the amino (N-terminus) of the next amino acid in line
3. Forms a peptide bond
4. Water is released as a byproduct of this reaction (OH from the acid and H from the amino)
5. Describe two primary structural components of polypeptide chains
6. Uncharged, rigid backbone, high hydrogen bonding potential
7. Variable side chains
8. Distinguish the difference between cis and trans peptide bonds and why the trans is preferred
9. A line drawn along the axis of the peptide bond (C---N) will separate the R side chains
10. If on the same side of the line then they are cis (highly unfavored due to steric hinderance)
11. If on opposite sides of the line then they are trans (highly favored, most common form)
12. Define what dihedral angles refer to and what restricts them as referred to by a Ramachandran diagram
13. Since the peptide bond is not a true double bond, some rotation is possible
14. The angle that the N can “spin” with its attached groups acting like a propeller and the C being the nose of the airplane in this case is the Phi ɸ angle
15. The angle that the C can “spin” with its attached groups now the propeller and the C of the carboxylic acid being the nose of the airplane is the Psi ψ angle

\*\* REMEMBER pHi comes before pSi so in the convention of N to C terminus this will work ☺

1. These angles are restricted by steric hinderance
2. Define why a polypeptide chain is polar
3. All polypeptide chains are polar because they are asymmetrical and have polar atoms giving rise to polarity
4. Explain the biochemically reversible reaction: cysteine—cysteine
5. The Cys-Cys bond is a covalent linkage of two cysteine residues on a single polypeptide or between polypeptides
6. During this linkage, the H atoms on each cysteine are removed (oxidation) and the sulfur atoms covalently linked to each other in a disulfide bond S-S
7. This reaction is reversible and mediated by enzymes, reduction of the cysteines (adding H back) results in the link being broken
8. Given the mass (kD) of a protein, be able to determine the probable number of amino acids it contains or vice versa
9. 1 Dalton is the mass of a hydrogen atom
10. The average mass of an amino acid is 110 daltons
11. Therefore to determine the number of amino acids in a protein divide the Daltons by 110 and you will have an approximate number of amino acids, reverse this process to determine the weight of a polypeptide of known length
12. What are structural differences between alpha helixes and beta strands

|  |  |
| --- | --- |
| Type of Structure | |
| Alpha Helix | Beta Sheets |
| Right handed (almost all)  ~1.5 Å wide  3.6 residues per turn  \*\*3.6x100=360°=1 turn ☺  Stabilized by H bonding to AA 4 residues apart  (i.e. #1 bonds to #5)  All backbone N-H and C=O are H-bonded | Bonded sheets ~3.5 Å apart  R groups are above and below the peptide bonds  Can be parallel or anti-parallel  Parallel sheets have N-H and C=O of one AA bonded to TWO different AAs in the other chain (bonding occurs at #1 and #3, skips a AA in between)  Anti-parallel sheets have the N-H and C=O of one AA bonded to the C=O and N-H of ONE AA of the other chain  More structurally diverse  Most adopt twisted shapes |

\*AA=Amino acid

1. Explain the differences between a parallel and anti-parallel beta sheet

Answered in above table

1. Describe how reverse beta turns are stabilized in protein secondary structure
2. Hydrogen bonding stabilizes these sharp “hairpin” turns
3. Amino acid #1 bonds to amino acid #4 in the chain (i.e. skip 2 amino acids for the turn that do not hydrogen bond)
4. What is protein secondary structure composed of
5. Alpha helixes
6. Beta sheeta
7. Reverse turns
8. What rules are important for tertiary structure
9. Thermodynamic stability
10. Van Der Waals interactions
11. Generally speaking, hydrophilic side chains are on the outside where they can have favorable interactions with water and hydrophobic side chains are sequestered in the center where they can have favorable interactions with themselves and away from the water molecules

Cell Structure and Function

1. Compare the structure and function of microtubules, microfilaments and intermediate filaments

|  |  |  |  |
| --- | --- | --- | --- |
| Type of Filament | | | |
|  | Microfilament | Intermediate Filament | Microtubule |
| Function | Muscle contraction  Movement of bacteria/viruses  Changes cell shape  Microvilli  Cell attachment  (actinin, talin, vinculin) | Links to plasma membrane, microtubules and microfilaments  Structural support  Deformable framework  Adaptable connection between membrane and cytoskeleton  Anchor nucleus | Movement of vesicles and organelles  Central structure of cilia and flagella  Chromosome movement during mitosis |
| Structure | ~6-7 nm  G-actin monomers | ~10nm  Various proteins | ~25nm  Tubulin dimers  (α,β subunits) |

1. Explain the mechanism of movements performed by the assembly and disassembly of actin filaments and microtubules
2. Assembly
3. Begins with G-actin monomers
4. The monomers nucleate (form clumps) this is the rate limiting step
5. Two separate but intertwined filaments form (called F-actin at this point)
6. Elongation of the budding microfilament (requires above threshold G-actin concentration, Mg2+ and ATP)
7. Disassembly
8. Induced by the action of various proteins
9. Cofilin binds 2 actin monomers in a filament and causes destabilization leading to disassembly
10. Capping proteins bind to the ends and block further elongation of the filament (Cap Z: + end, Tropomodulin: - end, stabilizes it, Gelsolin: + end, also severs filament)
11. Describe the structure and function of the intermediate filaments

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Intermediate Filaments | | | | | | |
|  | Cytoplasmic | | | | | Nuclear |
|  | Keratins | Desmin | Vimentin | Glial Fibrillary Acidic Protein (GFAP) | Neurofilaments | Lamins |
| Location | Hair  Nails  Skin | All muscle cells | Embryo  Fibroblasts  Luekocytes  Endothelial cells | Astrocytes  Schwann cells  Oligodendroglia | Nuerons | All nucleated cells |
| Function | Tissue strength  Integrity | Sarcomere organization  Integrity | Structural integrity | Structural integrity | Axon organization | Nuclear structure and organization |

1. Describe the structure and function of microtubules
2. Structure
3. Tubulin dimers α and β
4. Polarized (+ and – ends) + ends have exposed β subunits and – ends have exposed α
5. Bind GTP
6. – end anchored in Microtubule Organizing Center (MTOC) also called a centrosome
7. Can be found in the cytoplasm as single hollow tubes of 13 protofilaments
8. In cilia and flagella as 9 peripheral doublets (13 protofilaments in one, 10 in the second) surrounding a pair of singlets (traditional 13 protofilaments)
9. In basal bodies and centrioles as triplets (13+10+10)
10. Function
11. Movement of vesicles and organelles within a cell
12. Cilia and flagella for coordinated movements
13. Chromosome movement and mitosis
14. Compare the properties of actin and tubulin

|  |  |
| --- | --- |
| Fiber Type | |
| Actin | Tubulin |
| 42 kDa  Monomer/globular  1 ATP bound/monomer  2 parallel intertwined strands  ~6nm diameter | 50 kDa α subunit  50 kDa β subunit  Globular/dimer  1 GTP bound/ dimer  Hollow tube of 13 protofilaments  ~25nm diameter |

1. List tubulin binding drugs \*\*\*Actin binding drugs listed here also

|  |  |  |  |
| --- | --- | --- | --- |
| Drug | Actin | Tubulin | Mechanism of Action |
| Colchicine  Colcemid |  | XXX | Inhibits addition of tubulin dimers leads to de-polymerization |
| Vinblastine  Vincristine |  | XXX | Induces aggregate formation of tubulin |
| Taxol |  | XXX | Stabilizes tubulin prevents normal  De-polymerization |
| Cytochalasins | XXX |  | Bind to microfilament ends and prevent polymerization |
| Phalloidin | XXX |  | Binds microfilaments and stabilizes them  Prevents normal  de-polymerization |

\*\*\*CyTochAlasIN binds ACTIN ☺ the other “C” drugs bind tubulin

\*\*\*VinBlastine as well as the other “V” drug, vincristine bind tuBulin

\*\*\*Taxol the Tubulin binder stabilizes, its similar acting drug on Actin is Phalloidin

(to remember which stabilizes and which destabilize)

(All “C” drugs prevent polymerization which leads to eventual DE-polymerization)

\*\*\*C comes first then D give a “C” drug get De-polymerization ☺

1. Review structures and functions of the interphase nucleus
2. Structure
3. Nuclear envelope
4. Nucleolus
5. Non-membrane bound
6. Observed only in interphase
7. Site of rRNA transcription, processing and assembly of ribosomal subunits
8. 3 regions
9. Fibrillar Center: stains pale, non-transcribed DNA
10. Dense Fibrillar Components: nucleolar RNA transcribed here
11. Granular Component: mature rRNA subunits assembled here
12. Chromatin
13. Heterochromatin
14. Condensed, inactive form
15. Euchromatin
16. Active form, DNA transcribed
17. Lamins providing structural support
18. Dysfunctional Lamin A causes PROGERIA
19. Continuous with rough ER
20. Contains Nuclear pore complexes (NPR)
21. Small macromolecules can diffuse
22. Larger ones require transport
23. Exportins export FROM nucleus
24. Importins import TO nucleus
25. Regulated by the Ran GTP-binding proteins
26. Define heterochromatin, euchromatin, centriole and centrosome
27. Centriole- components of the centrosome, 2 oriented at right angles make a centrosome
28. Centrosome- larger overall structure of centriole pairs

\*\*Heterochromatin & Euchromatin described above

1. Review the mechanism of chromosome movement during mitosis
2. Centrioles replicate during S phase
3. Are located at opposite ends of the soon to be dividing cell by M phase
4. Astral MTs used to orient the spindle network, hook into cytoskeleton
5. Kinetochore MTs launched from the MTOC hook into the kinetochore of the chromosomes lined up along the divisional plate
6. Inter Polar MTs attach spindle poles to each other but do not contact the kinetochore, interact with complementary Polar MTs on the other side forming anti-parallel MTs, dyneins and kinesins attach to these to help pull the two cells apart

Blood

1. Describe the composition of blood and how plasma and serum differ
2. Describe the cellular components of blood
3. Describe how hematocrit is calculated and how hematocrit values are interpreted
4. Hematocrit- the packed red blood cells per unit volume of blood
5. Normal Values:
6. Men 40-50%
7. Women 35-45%
8. Normal Hemoglobin and RBC numbers
9. Men—Hemoglobin 13.6-17.2 g/dL; RBC 4.3-5.9 million/mm^3
10. Women—Hemoglobin 12.0-15.0 g/dL; RBC 3.5-5.0 million/mm^3
11. Calculated by collecting whole blood, centrifuging to compact the cells and determining the percentage of the volume occupied by red blood cells which would be at the bottom

\*\*Cause for rejection: clotted or hemolyzed blood

(don’t know if this is important but it keeps popping up)

1. Recognize a RBC abnormality
2. Hereditary Spherocytosis
3. Rounded cells lacking the biconcave nature, resemble beach balls
4. Describe the different types of white blood cells and their properties
5. Describe the structure and function of platelets
6. Formed from megakaryocytes (giant polyploid cells in bone marrow)
7. No nucleus
8. Contain organelles
9. Have alpha, delta and lambda (lysosome) granules
10. “Pinched” off from megakaryocyte membrane
11. Required to coagulate blood
12. Disk like structure maintained by microtubules
13. Describe the similarities and differences between humoral and cell mediated immunity
14. Describe the 5 classes of antibodies and the differences in their function
15. Describe one example of abnormal function of white blood cells
16. HIV infection causes destruction of Helper T-Cells

Plasma Membrane 1

1. Describe 3 common types of membrane lipids and discuss the amphipathic nature of each. Indicate how membrane lipid structure facilitates self-assembly of the lipid bilayer
2. Phospholipids- have long chain fatty acids (hydrophobic) and a polar head group (phosphate)
3. Glycolipids- have long chain fatty acids (hydrophobic) and a polar head group (sugar)
4. Cholesterol- has the hallmark 4 fused ring backbone (hydrophobic) and a single polar hydroxyl (OH)
5. This structure, being mainly hydrophobic tends to aggregate so as to minimize contact with water, however, the polar heads being hydrophilic like to face the water/lipid molecule interface, this sequesters the hydrophobic portions together and the hydrophilic portions end up on the outside in a bi-layer (otherwise water would have to interact with the lipid chain on the other side, a monolayer would not be useful for a cell because it could have no cytoplasm)
6. Explain what types of molecules can pass directly through the lipid core of membranes and which cannot and why
7. Small molecules such as oxygen and carbon dioxide can readily diffuse through the membrane because they don’t interact/come in contact with the actual lipid core
8. Small polar molecules like ethanol and water (depending on cell) can also pass through also because of their small size will have little interaction with the lipid core
9. Large uncharged polar molecules (glucose) cannot pass
10. Ions cannot pass (Na+, Cl-)
11. Charged polar molecules cannot pass (amino acids)
12. Discuss the distinguishing features of integral and peripheral membrane proteins using glycophorin as an example

|  |  |
| --- | --- |
| Type of Membrane Protein | |
| Integral | Peripheral |
| Tightly associated with plasma membrane  Requires harsh conditions (detergents) to remove for scientific analysis  Enters and/or spans the hydrophobic core  (usually contain alpha helixes if protein is spanning membrane) | Loosely associated with the membrane  Mild conditions will dissociate these proteins  (salts, pH)  Do not enter or span hydrophobic core |

\*\*Glycophorin is an integral protein (transmembrane) it utilizes alpha helixes to span the plasma membrane in red blood cells

1. Explain how lipids can serve to anchor some proteins in the plasma membrane
2. Having a molecule that is hydrophobic on one end and polar on the other (similar to membrane lipids) allows part of the molecule to embed in the plasma membrane while the hydrophilic end is free to make covalent attachments to proteins that need to be near the plasma membrane for optimum functioning but otherwise could not remain there on their own
3. Explain the impact of fatty acid length and saturation, and the effects of cholesterol on membrane fluidity as reflected by the melting temperature (Tm)
4. Melting temperature increases as length of fatty acid chains increases (this is due to the increased hydrophobic interactions between chains)
5. Introducing unsaturation (double bonds) causes kinks in the side chains which prevent them from packing neatly and thus causes less than optimal interactions to occur (this lowers the melting temperature)
6. Cholesterol exhibits a dual effect on plasma membranes depending on the external conditions
7. If the cell is stressed with lower temperatures (normally leading to decreased fluidity) cholesterol acts as a giant “kink” preventing the fatty acid chains from packing together neatly as they would normally do when temperature decreases
8. However, if the cell is stressed with warmer temperatures (normally leading to increased fluidity) cholesterol with its large, bulky, planar rings actually prevents too much motion by acting like a speed bump, molecules must go around cholesterols large rings
9. Discuss one example of asymmetry of membrane lipids and membrane proteins. For example the impact of exoplasmic phosphatidyl serine on cell destruction or the asymmetric nature of glycosylation on membrane proteins
10. Cells do not display the same proteins on the cytosolic surface as they do on the extracellular surface
11. Phosphatidyl serine on the extracellular surface signals macrophages to engulf red blood cells in the spleen
12. Amino containing phospholipids are mainly found on the cytoplasmic side while glycosylated lipids are mainly found on the extracellular side along with choline phospholipds
13. Discuss the types of interactions among the key protein components of red blood cell membranes. Describe their contribution to its strength and flexibility in health and disease, for example hereditary spherocytosis
14. Major proteins in the RBC that contribute to its incredible flexibility and strength
15. Glycophorin (intergral protein)
16. Band 3 an anion exchange protein (integral protein)
17. Ankyrin (peripheral protein)
18. Spectrin (peripheral)
19. Actin (peripheral)
20. Mutations in the genes encoding spectrin or ankyrin lead to weakened interactions between peripheral and integral membrane proteins
21. These cells are osmotically fragile (do not respond well to changes in solute gradients)
22. Hereditary spherocytosis

Epithelial Tissue

1. Identify the two major subdivisions of the epithelium (epithelium and glands) and their embryologic origin
2. 2 divisions are epithelia and glands
3. Derived from all 3 germ layers
4. Endoderm (inner most layer)- gives rise to linings of the G.I. tract, respiratory tract and distal parts of the urogenital tract
5. Mesoderm (middle layer)- gives rise to linings of the internal cavities

When lining the blood vessels and heart it is called endothelium (although not derived from the endoderm itself)

When lining the body cavities (peritoneum, pericardium, pleura) it is called mesothelium

1. Ectoderm (outermost layer)- gives rise to the epidermis
2. Be able to define the tissue epithelium, list its various functions and its common structural characteristics
3. Epithelium- a layer of cells that covers the body’s surfaces, provides a selective barrier to aid or prevent materials from traversing into the areas they cover
4. Structure
5. Predominantly cellular
6. Numerous intercellular junctions
7. Polar cells (have distinct basolateral and apical portions that have different functions)
8. One boundary of any epithelial cell is ALWAYS a free surface
9. Rests upon the basement membrane or connective tissue
10. Avascular (has no blood vessels running through it) nutrients diffuse from underlying
11. basement membrane
12. Function
13. Selectively permeable barrier
14. Protection from mechanical, chemical and dehydration stresses
15. Secretory layer (glands are included in this layer)
16. Absorption (G.I. tract)
17. Transport (lungs, exchange CO2/O2)
18. Sensory surface (lets brain know what is going on in the outside world)
19. Regenerative and self-repairing tissue
20. Be able to describe and classify the various types of epithelium by cell shape and number of cell layers
21. Classified based on number of layers and shape of cells
22. Number of layers
23. Simple/Unilaminar (one layer)
24. Stratified/Multilaminar (multiple layers)
25. Cell shape
26. Squamous (thin flattened cells)
27. Cuboidal (resembles cubes, height~width)
28. Columnar (resembles columns, height>width)
29. Be able to describe the polarity of the epithelial membrane and how its specializations contribute to its polarity and function. Be able to relate other structural specializations to epithelial functions
30. Two domains of epithelial cells, specialized to carry out different functions
31. Apical domain
32. Contacts the free surface
33. Plasma membrane has carrier proteins (for transport inward)
34. Glycoproteins (recognition) forms glycocalyx (fuzzy coating of cells)
35. Hydrolytic enzymes
36. Aquaporins (facilitated diffusion of water)
37. Capable of endocytosis, exocytosis and transcytosis (transport of materials to basolateral surface)
38. Clathrin protein is present here which helps in forming vesicles during endocytosis
39. Basolateral domain
40. Consists of lateral sides and base of cell
41. Characterized by the junctional complex (site of cell-cell adhesion)
42. Junctional complex formed from 3 main structures
43. Zonula occludens (most apical)

I. prevents passage of materials from apical membrane to basolateral

II. fuses adjacent cells to each other using transmembrane proteins like claudin and occludin, reinforced by cadherin

III. Forms a tight junction, defines the barrier between apical and basolateral domain

1. Zonula Adherens

I. Forms a mechanical attachment to adjacent cells

II. Uses the transmembrane linker protein Cadherin which binds to the cytoskeleton along with other proteins such as vinculin, actinin, and talin (these bind actin filaments to cell membrane linking cells together through their cytoskeletons)

1. Macula Adherens

I. Deepest junction (closest to basolateral membrane)

II. Another mechanical attachment site

III. Looks like a spot weld or bulge in the cell due to dense attachment plaques desmoplakin and plakoglobin

1. Antibodies are produced to the transmembrane cadherins that attach the dense plaques on the cytoplasmic side of each cell to each other in the autoimmune disease Phemphigus Vulgaris, this causes sloughing of the skin and other epithelial layers
2. Describe and differentiate between an exocrine and endocrine gland
3. Exocrine glands have connections to the free surface through ducts, their secretions are placed on the apical surface of the epithelia
4. Endocrine glands have no connection to the free surface and instead secrete their products into the extracellular matrix surrounding the epithelia
5. Be able to describe the common components of an exocrine gland and classify exocrine glands by morphology, mode of secretion and type of secretory product
6. Morphological classification
7. Unicellular- single cells embedded in epithelium that excrete directly onto surface (goblet cells excreting mucous)
8. Multicellular
9. Numerous cells that can be divided into a “duct” and “secretory” portion
10. Named according to branching of duct system then shape of secretory portion
11. Duct is either simple (one duct) or compound (multiple branching ducts)
12. Secretory portion is either tubular (long column) or alveolar/acinar (lobe/grape shaped)
13. By mode of secretion
14. By type of secretory product
15. Mucous- secretes a thick and viscous solution rich in mucinogens
16. Serous- secretes a watery solution possibly rich in ions or enzymes
17. Mixed- secrete solutions that are a combination of mucous and serous secretions
18. Sebaceous- secrete an oily solution rich in lipids
19. Ceruminous- secretes thicker waxy substance mainly used for protection
20. Be able to define and describe the different levels of organization in the body
21. Cells- basic unit of all living systems
22. Tissues- group of similar cells working together to perform a specific function
23. Organs- two or more different tissues coming together to perform a single function
24. Systems- two or more organs coming together to perform a single function
25. Be able to list and describe the four basic tissue types of the body
26. Epithelium- layer of cells covering the body’s surfaces
27. Muscle- group of cells that change shape and are specialized for contraction
28. Nerve- cells specialized to conduct electrical impulses, receive stimuli and carry that information to and from the brain
29. Connective- cells that provide the framework for all other cells of the body, integrates and connects all tissues, all blood vessels are contained within connective tissue, relatively few cells but large amounts of matrix between them

Introduction to Proteins: Part 3

1. Within an aqueous environment was is the expected characteristics of a globular protein’s exterior amino acid composition and of its interior amino acid composition
2. Exterior- mainly composed of hydrophilic amino acids
3. Interior- mainly composed of hydrophobic amino acids
4. Define a protein domain
5. Domain- a compact globular region of a SINGLE protein chain connected to other domains by a flexible linker region
6. Define a subunit of a quaternary structure
7. Subunit- an ENTIRE polypeptide chain (may contain multiple domains) that was separately synthesized and later bonded covalently or otherwise to another polypeptide chain to form a functioning protein
8. What does β-mercaptoethanol and 8M urea do to proteins and how can the effects become reversed
9. β-mercaptoethanol- potent chemical disrupter of disulfide (Cysteine-Cysteine) bonds in proteins
10. Urea- organic compound that in high enough concentrations (8M) will disrupt non-covalent (ionic, hydrogen bonding, van der waals interactions) in proteins
11. These compounds would be classified as “denaturants” as they disrupt the normal conformation adopted by a functional protein and generate an inactive/misfolded protein
12. Reversal of these effects is simple enough in that removal of the denaturing chemicals will allow a protein to re-fold into its native conformation \*\*
13. Both chemicals must be removed at the same time otherwise the protein does not adopt its native conformation
14. Removing β-mercaptoethanol will allow the protein to reform disulfide bonds but with 8M urea remaining the disulfides that form will likely not be the correct
15. Removing only urea but keeping the β-mercaptoethanol will allow the protein to fold but not give it the ability to form disulfide bonds necessary for proper configuration, this will also lead to a misfolded protein

\*\* Not all proteins can do this, some will remain denatured even after the chemicals have been removed because they needed special proteins like chaperones to adopt their native conformation

1. List two factors that facilitate protein folding and describe the importance of cooperative transition
2. Cooperative Transition
3. As a protein is folding into its native conformation, it is observed that a rapid, almost instantaneous transition occurs between a completely un-folded protein and one that is in the native conformation
4. Process is referred to as being “all or none”
5. Protein “snaps” from folded to unfolded rather suddenly
6. Cumulative Selection
7. As a protein is folding, portions of the polypeptide chain that are folded correctly are preserved while the rest of the protein attempts to find its proper configuration

\*\* Don’t know if anyone else noticed this but these two seem to be contradictory to me. How can a protein be 100% folded or 100% unfolded with a rapid transition as is described by cooperative transitioning yet have parts folded correctly and maintained while the rest of the protein folds as is described by cumulative selection?? If anyone has any insights it would be appreciated

1. How do knowledge based methods provide insights into the 3-dimensional conformation of proteins of known sequence
2. Knowledge based methods use the amino acid sequence of previously known structural conformations of proteins to “predict” the conformation an unknown protein with a known sequence will adopt
3. Similar to figuring out what someone may be cooking for dinner based on their grocery list and a list of known recipes (usually a good approximation but definitely not absolute)
4. Explain what a prion is and how it can cause CJD
5. Prion- self propagating forms of chromosomally encoded protein
6. Normal prion proteins are α-helical in structure but for unknown reasons some of these proteins adopt the β-pleated sheet conformation
7. Once a prion protein becomes a β-sheet it mysteriously causes normal prion proteins to adopt the β-sheet conformation
8. Once enough prion proteins are in the β-sheet conformation, they aggregate and form insoluble globules which rapidly lead to CJD
9. Describe the different types of covalent post-translational modifications that occur to proteins and subsequent results
10. Phosphorylation
11. 3 amino acids commonly receive this type of modification
12. Serine🡪 phosphoserine
13. Threonine🡪 phosphothreonine
14. Tyrosine🡪 phosphotyrosine
15. Hydroxylation
16. 2 amino acids commonly have this addition
17. Proline🡪hydroxyproline
18. Lysine

\*Necessary for strength of collagen, prolyl hydroxylase performs this function and requires ascorbic acid (Vitamin C) without it the disease Scurvy (common with sailors back in the day) will develop

1. Acetylation
2. Acetyl group bonded to amino terminus which helps resist degradation by enzyme
3. Modification used in histone wrapping of DNA
4. Describe the first two essential steps that must be taken when exploring an unknown cellular protein for function
5. Purify the protein of interest
6. Need to have pure protein to determine the amino acid sequence
7. Help determine evolutionary relationships
8. Determine biochemical function
9. Structure from x-ray crystallography
10. Determine the primary amino acid sequence
11. Describe when you would choose affinity chromatography rather than ion exchange or gel filtration during protein purification
12. Pretty much whenever possible, I can’t really see a downside to it other than it might not be feasible, other than that I put this together

|  |  |  |
| --- | --- | --- |
| **Separation Type** | **Property Used to Separate** | **Use/Benefits** |
| Centrifugation | Mass | Initial separation from cell lysate |
| Dialysis | Solubulity  Size | Removal of small particles from larger ones |
| Column Chromatography  (Gel filtration) | Size | Separation of a wide range of particles |
| Affinity Chromatography | Binding affinity | Highly specific  \*best method |
| Ion Exchange | Charge | Separation of positive from negatively charged particles |
| High Pressure Liquid Chromatography  (HPLC) | Affinity  Size | Advanced form of chromatography increased resolution  quick data |
| SDS-PAGE | Size | Easy determination of purity |

1. Explain why SDS-PAGE is important to determine success of protein purification steps
2. SDS-PAGE separates proteins based on their size/molecular mass
3. If purification steps are successful there should only be one band in the gel corresponding to a protein of a specific size and no others
4. Additional bands usually indicate contamination with other proteins
5. Explain why protein specific activity is important during protein purification
6. Protein specific activity is another way besides SDS-PAGE to determine the success of the purification, the better purification the more “active” the sample becomes relative to mass

Plasma Membrane 2

1. Distinguish the major kinetic differences between simple and facilitated diffusion
2. Simple Diffusion
3. Only allowed for small neutral molecules (O2, CO2) or small polar molecules

(H20, ethanol)

1. Always down the concentration gradient
2. Facilitated Diffusion
3. Ion Channel Mediated
4. Extremely high rate of diffusion
5. Most are “gated” (i.e. respond to specific signals either ligand, voltage change or mechanical stress) \*Exception is the “leaky” K+ channel which is always open
6. Specific for certain ions, protein channel contains a Selectivity Filter (narrow portion of ion channel that excludes hydrated ion shells that do not have a specific low energy interaction with the amino acids of the inner channel, called “shedding”)
7. Repulsive forces push ions through the channel
8. Voltage Gated (Na+/K+)
9. Ligand Gated (Nicotinic Acetylcholine Receptor)
10. Carrier-Mediated

1. Much higher rate than passive (simple) diffusion

2. Selective transport

3. Observes saturation kinetics (i.e. there are only so many receptors)

4. Increases the rate of thermodynamically favored diffusion

5. GLUT transporters are an example of this (GLUT4 is the only insulin responsive one)

1. Compare the ion concentrations in the ECF and ICF, and discuss the role of these electrochemical gradients in membrane transport
2. Important Ion Concentrations in Extra Cellular Fluid (ECF)/Intra Cellular Fluid (ICF)

|  |  |  |
| --- | --- | --- |
| Fluid Type | | |
| Ion/Molecule | ECF (mM) | ICF (mM) |
| Na+ | 140 | 10 |
| K+ | 4 | 140 |
| Ca++ | 2.5 | 0.0001 |
| Glucose | 5.5 | ~1 |

1. Without these differences in ion concentration cells could not function, produce ATP, propagate action potentials or contract muscles
2. Describe the mechanism of action of GLUT-4 in the insulin responsive glucose uptake
3. Meal high in carbohydrates is consumed
4. Glucose is transported across the epithelial lining of the intestines into the blood
5. β-cells of the pancreas sense increased blood glucose
6. Insulin is secreted into the blood
7. Insulin responsive cells (muscle, adipose) are induced through binding of the insulin receptor to translocate GLUT4 sequestered within the cell to its plasma membrane
8. GLUT4 uptakes glucose from blood lowering blood glucose levels
9. Describe how the Na+/K+ ATPase primary active transporter functions to drive secondary transport of glucose. Understand symporter antiporter concept
10. Na+/K+ ATPase hydrolyzes ATP to pump Na+ outside the cell (against its concentration gradient) and K+ into the cell (also against its concentration gradient)
11. This creates an electrochemical potential voltage across the plasma membrane
12. This gradient is used by the Na+/Glucose symporter which allows Na+ back into the cell down its concentration gradient while “pulling” a glucose along with it against its concentration gradient
13. Explain the mechanism of action of cardiotonic steroid drugs on the Na+/K+ ATPase
14. The Na+/K+ ATPase uses phosphorylation/dephosphorylation to regulate its cycle
15. Cardiotonic drugs are designed to indirectly increase cardiac contraction strength by acting upon the Na+/K+ ATPase
16. This is achieved because cardiotonic drugs inhibit dephosphorylation of the Na+/K+ ATPase preventing it from returning to an “open” conformation after bound K+ is released
17. This directly affects the levels of Na+ in the cell causing it to rise
18. However, the cell compensates by running the Na+/Ca++ transporter (normally exporting Ca++ and importing Na+) in reverse there by relieving the stress of increased intracellular Na+
19. This action increases the Ca++ within a cell which the indirectly increases contraction strength in cardiac tissue
20. Explain the structural basis of the ion selectivity of the voltage gated K+ channel
21. The ion channels are shaped like a funnel with a large opening at the surface but a narrow opening in the middle, this is the basis for selectivity
22. The narrowing of the opening is called the Selectivity Filter
23. At this point, ions cannot pass in the hydrated form they exist as in the extracellular fluid
24. In order to pass through the channel and down their concentration gradient they must part with the surrounding water molecules in a process called “shedding”
25. In order for an ion to “shed” its water shell, it must make energetically favorable interactions with the surrounding amino acids in the channel, if this occurs then the ion can pass, if “shedding” creates less favorable interactions the ion does not do so and thus is prevented from passing
26. In this way ion channels can “select” for the specific ions they wish to allow through
27. Describe the involvement of ABC-type ATP powered pumps in cystic fibrosis and multidrug resistance
28. Cystic Fibrosis
29. Cystic fibrosis is caused by a mutation in the Cystic Fibrosis Transmembrane Regulator (CFTR) an ABC-type ATPase
30. This mutation prevents CFTR from being transported to the plasma membrane where it would normally act to export Cl- from within the cell
31. With CFTR non-functional, Cl- builds up inside the cell causing Na+ to flow inward to balance the increasingly negative charge within the cell
32. As Na+ flows in, the solute concentration within the cell increases causing water to flow inward through osmotic pressure
33. The inward flow of water dehydrates the surrounding mucous layer in the epithelial cells lining the respiratory tract leading to thick mucous that is difficult for the cilia to remove
34. This mucous traps bacteria without removing them and eventually leads to chronic respiratory tract infections
35. Multidrug Resistance 1 (MDR1)
36. MDR1 is an ABC-type ATPase
37. It functions to pump small planar drugs out of cells
38. These happen to be the very types of drugs used to treat a wide variety of diseases like cancer, malaria and bacterial infections
39. This ATPase prevents effective treatment using these compounds by pumping the drug back out of the cell before its concentration reaches the threshold necessary to exert its effect
40. Describe the role of the acetylcholine receptor in a channelopathy
41. Myasthenia Gravis is an autoimmune disease characterized by production of antibodies to the acetylcholine receptor present in the neuromuscular junctions (NMJ)
42. Antibodies bind to and inactivate the receptor thus when an action potential signals the release of acetylcholine into the NMJ it is unable to bind its receptor and therefore cannot cause depolarization in the postsynaptic cell
43. Acetylcholinesterase however is functioning normally and rapidly degrades acetylcholine in the NMJ
44. Acetylcholinesterase inhibitors can be used to increase the length acetylcholine remains in the NMJ and give it a longer opportunity to bind in the presence of the antibodies

Cell Cycle

1. Describe the phases of the cell cycle and the stages of mitosis
2. Cell Cycle
3. Interphase (G1-S-G2)

1. G1- normal cell volume, diploid number of chromosomes, synthesis of replication machinery (DNA polymerase, regulatory proteins and various other enzymes)

2. S- DNA replicated, by the conclusion of S phase cell will have double the normal amount of DNA (4N)

3. G2- Synthesis of proteins necessary for mitosis, DNA “spell checked” for errors and corrected if found, cell contains 2 complete diploid sets of chromosomes throughout this phase

1. Mitosis (M)
2. Prophase- chromosomes condense and are visible for the first time, nucleolus disappears, mitotic spindle formation outside nucleus, kinetochore formation
3. Pro-metaphase – breakdown of nuclear envelope due to lamin phosphorylation, chromosomes attach to mitotic spindle using kinetochore, random arrangement of chromosomes at this stage
4. Metaphase- chromosomes aligned in middle of cell along plane of division, kinetochore microtubules attach sister chromatids to opposite poles of spindle
5. Anaphase- sister chromatid separation to form two daughter chromosomes, kinetochore microtubules get shorter and spindle poles move apart
6. Telophase- daughter chromosomes arrive at their respective poles and begin to decondense, formation of a new nuclear envelope, formation of contractile ring
7. Cytokinesis- cytoplasm divided in equal portions by contractile ring composed of actin and myosin filaments, after completion of this 2 new cells each with a nucleus and the diploid number of chromosomes remain
8. Summarize the roles of cyclins and cyclin dependent kinases (CKDs) and other interacting proteins in the regulation of the cell cycle

\*\*Cyclins and CDKs lined up to match which act together

1. Understand how oscillations in the activity of the cyclin-CDK complxes occur and how they regulate the timing and directionality of the cell cycle
2. When a cell receives certain mitogenic (pro mitosis) signals E2F to facilitate transcription of the cyclins
3. Specific cyclins build up at specific points in the cell cycle
4. Cyclin D during G1 (activates CDK 4, 6) pushes cell past “restriction point”
5. Cyclin E during late G1 (activates CDK2) required for cell to progress to S phase
6. Cyclin A during S and G2 (activates CDK2) required for cell to pass through S phase
7. Cyclin B during late G2 and M (activates CDK1) required to enter and eventually leave M phase
8. Cyclins have a “cyclin destruction box”, which signals them for polyubiquitination and eventual degradation, this keeps the cell cycle moving in one direction
9. Explain what checkpoints are and how checkpoints work
10. Checkpoints are specific places in the cell cycle that a cell my arrest if there is a problem
11. These checkpoints are sensitive to various factors
12. G1 checkpoint (restriction point)- mitogenic signals/DNA damage
13. S checkpoint- incomplete replication
14. G2 checkpoint- DNA damage
15. M checkpoint- unattached kinetochore
16. Understand the roles that Rb and p53 play in checkpoint control
17. Rb
18. Inhibits cell cycle progression during G1 by binding the E2F transcription factor
19. In response to mitogenic signals, cyclin D is produced which complexes with CDK 4/6, this complex phosphorylates Rb and causes its dissociation from E2F thereby inducing cell cycle progression
20. p53
21. In response to DNA damage, p53 becomes phosphorylated and dissociates from its inhibitor MDM2
22. It goes on to prevent cell cycle progression during G1 by being a positive transcription factor for the CDKi p21 which when synthesized inhibits CDKs 2,4 and 6
23. Associate cell cycle defects with altered cell growth and division
24. Over-expression of cyclin D is linked to esophageal, breast and gastric cancer
25. CDKi mutations are linked to development of multiple tumor types
26. Loss of both alleles for Rb causes retinoblastoma tumors
27. Recognize the clinical significance of the uncontrolled cellular proliferation in cancer
28. Because cancer cells have lost the ability to arrest at checkpoints various treatments like chemotherapy and radiation are effective at destroying cancer cells
29. Chemotherapy uses toxic drugs targeted to rapidly dividing cells, although by no means specific to cancer cells, the theory is that cancer cells by dividing rapidly will be more effected by the drugs than healthy tissue
30. Radiation takes advantage of the fact that cancer cells cannot arrest once DNA damage has occurred, normal cells will repair this damage before proliferating while cancer cells will proliferate with damaged DNA ultimately leading to cell death